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Studies on *Conchophthirius mytili* De Morgan.
II. Conjugation and Nuclear Reorganization.

By

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(With 9 figures in the text and plate 5—7.)

Contents.

Introduction.

In the first of this series of studies I gave a description of the general morphology and division of *Conchophthirius mytili*, a commensal found in the mantle cavity of the common salt water mussel, *Mytilus edulus*. In the present paper I wish to describe the process of conjugation and the phenomena subsequent to conjugation in this species. *Conchophthirius mytili* offers excellent material for a minute cytological study of the changes that take place during and after conjugation, due to the large size of the nuclei and the relative flatness of the organism.

This work was carried on at the Zoological Laboratory of Columbia University in New York City.

I take pleasure in expressing my appreciation to Prof. GARY N. CALKINS for kindly encouragement and helpful criticism throughout the progress of this work.

Methods.

The ciliates used for this study were obtained from salt water mussels collected from the rock breakwaters of Brighton Beach, Coney Island, New York. The same technical methods were used as described in the first of this series of studies.

Of the many fixatives used the most satisfactory were sublimate-acetic in 95 % alcohol, SCHAUDINN'S, BOUIN'S and GILSON-CARNOY'S fluids.

The stains employed were HEIDENHAIN'S and DELAFIELD'S haematoxylins, the FEULGEN nuclear reaction and the BORREL stain.

For micronuclear study HEIDENHAIN'S haematoxylin and the FEULGEN nuclear reaction gave the best results, while the BORREL stain and the FEULGEN reaction were superior for the study of macronuclear changes.

A description of the formulae and details of technique for both the BORREL stain and the FEULGEN reaction are given by CALKINS (1930 a). I obtained very satisfactory results with the FEULGEN reaction by using the concentrations and times given by him. For the BORREL stain, however, the time of staining must be modified to suit each form, for the best results. I found I could get excellent preparations of exconjugants by staining for seven minutes in the BORREL I solution (saturated aqueous solution of Diamant fuchsin), rinsing in distilled water, staining for five minutes in the BORREL II

solution (3 parts saturated aqueous solution of indigo carmin and 2 parts saturated aqueous solution of picric acid) and passing the slides rapidly through the lower alcohols to 95 % without further washing. In this way just enough of the green acid stain was left in the cell. Differentiation of the red basic stain was watched under the microscope. This took from ten to thirty seconds. When differentiation was complete the slide was dipped several times in absolute alcohol, cleared in xylol and mounted in damar.

Occurrence.

Conjugation in *Conchophthirius mytili* seemingly takes place at rare intervals. Up to this time only one epidemic has been noted. Collections and examinations were made twice a week throughout the early fall of 1931 yielding only normal vegetative ciliates. On October 24, however, the first pair of conjugants was noted. This was fixed and stained. Collections were made four and five times a week from that date until November 20. On October 25 many conjugating pairs were found showing practically all stages of micronuclear activity. In the following few days a sharp decline in conjugation occurrence took place, and a relatively low percentage of conjugants were found thereafter. On December 6 the last conjugating pair was found. From October 25 on, most of the individual ciliates found were in some stage of reorganization. This was easily determined, even with the dissecting microscope, by the appearance of the nuclei.

As stated in the first of these studies it was impossible to keep the ciliates alive for more than a short period after their removal from the host. Under such conditions it was impossible to determine the length of time required for the processes of conjugation and reorganization. Judging from the comparative frequencies of occurrence I should say that the period of union was short, probably only a few hours, while the time for complete reorganization of the exconjugant must be several weeks.

The epidemic of conjugation came three of four days after the first freezing weather of the season. Whether or not this was a contributing cause to the epidemic is problemmatical. As the mussels are exposed at low tide, twice every twenty-four hours, they have ample opportunity to become thoroughly chilled. Further observations and experiments are planned with this question in mind.

Cytoplasmic structures.

The method of fusion of conjugating *Conchophthirius mytili* is very unusual. The anterior end of the peristomal region of one comes in contact with the aboral surface of the other well toward the anterior end. A rather wide protoplasmic bridge is formed between the two. This presents a picture of extreme asymmetry (Text-Fig. 1, Pl. 7 Fig. 30). The oral regions of both ciliates are exposed and apparently continue to function throughout the process. There is no evidence of a reorganization of the mouth

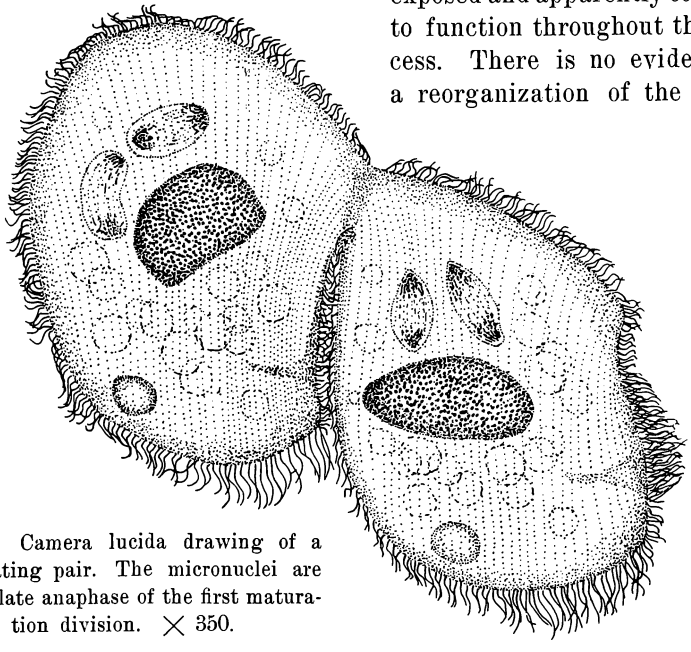


Fig. 1. Camera lucida drawing of a conjugating pair. The micronuclei are in the late anaphase of the first maturation division. × 350.

parts, as described in *Chilodon* by MAC DOUGALL (1925), during conjugation. This is put off until the first division of the exconjugant. At that time duplication and reorganization of the whole peristomal region takes place, as in normal vegetative division. The conjugants are usually loaded with food inclusions throughout the process.

There appears to be a distinct size difference in the conjugants, the larger one always being the ciliate which fuses in the region of its peristome. In some cases this difference is slight but in the great majority of cases it is well marked (Pl. 7 Fig. 30). This size difference is not unusual among ciliates, but the fact that a distinct differentiation exists whereby the larger gamont always fuses at its peristomal region is unique. MIYASHITA (1927) described for *Lada*

tanishi a somewhat similar case of peculiar fusion during conjugation. In this ciliate, a parasite of a Japanese snail, there is a size difference between the conjugants. The smaller conjugant ("microconjugant") attaches itself by its anterior (aboral) end to the ventral surface of the larger conjugant ("macroconjugant"). Both oral regions remain widely separated. According to STUDITSKY (1932) *Ptychostomum chattoni*, a parasite of *Lumbriculus variegatus*, conjugates in the same manner as *Lada*.

Nuclear phenomena.

The micronuclei of *Conchophthirius mytili* during conjugation conform, in general, to the usual ciliate type. A few minor differences may, however, take place.

The number of micronuclei taking part in the pre-zygotic divisions differs according to the number of micronuclei possessed by the preconjugants. Cases have been found where two uni-micronucleate, two bi-micronucleate and two tri-micronucleate organisms have conjugated. On the other hand many cases have been seen where the micronuclear number of the conjugants was different, e. g. a bi-micronucleate conjugating with a tri-micronucleate organism. In any case degeneration of some of the products of the first, second and third maturation divisions results in the formation of only two pronuclei per conjugant.

As a typical case I shall outline the usual procedure in one conjugant when two tri-micronucleate organisms conjugate.

The first maturation division results in six apparently equal nuclei. Two or more of these degenerate and the remaining ones divide again. Of the result of this second maturation division usually only two, rarely three or four, remain, the others degenerating. These two, three or four micronuclei complete the third division, degeneration of all but two of the products taking place rapidly. These two are the pronuclei. The exchange of the pronuclei is followed very shortly by the separation of the conjugants. A diagram of this process is found in Text-Fig. 5 A—H.

First maturation division.

The earliest stages of micronuclear activity that I was able to find show the chromatin collected in the center of the swollen nucleus in a finely granular mass. The FEULGEN nuclear reaction shows this mass as a weakly staining core in a colorless matrix.

With HEIDENHAIN'S haematoxylin fine fiber-like strands can be seen emanating from the core and running in an irregular manner to the periphery of the nucleus (Pl. 5 Fig. 1). This chromatin mass then spreads out into a lightly staining, rough plate across the nucleus, and spindle fibers are seen to have formed. Whether or not these spindle fibers are the same fibers as were seen in the earlier stage I can not tell. During this time the whole nucleus has increased in size, now measuring 17 μ to 20 μ on its long axis (Pl. 5 Fig. 2). At this stage the micronuclei are easily visible in life under low magnifications. The fine chromatin granules gather to form larger granules, numbering about thirty-two (thirty to thirty-four) in a typical metaphase plate, the spindle fibers now being quite distinct (Pl. 5 Fig. 3). I have never been able to see any evidence of division centers at the converging ends of the spindle fibers. In all cases the fibers seem to end abruptly, forming a very blunt spindle. The nucleus has now reached a maximum size, measuring 18 μ to 24 μ on its long axis. The early anaphase shows a sorting out of the granules (Pl. 7 Fig. 31) into two sets of approximately sixteen each (Pl. 5 Fig. 4). Each set of daughter granules migrates toward its respective pole of the spindle and becomes irregularly drawn out (Pl. 5 Fig. 5). I have no material showing the constriction and separation of the daughter nuclei.

My observations on the prophase stages of this first division are obviously not complete. The parachute type, as has been described in *Uroleptus mobilis* (CALKINS, 1919), *Oxytricha fallax* (GREGORY, 1923) and many others, and the crescent stage, as in *Paramaecium* (CALKINS and CULL, 1907) are lacking in *Conchophthirius mytili*. This may be explained by a probable short duration of a corresponding stage which was missed. The alternative, of course, is to assume that there is no stage in this ciliate comparable to either the parachute or the crescent stage. This is apparently the situation found in *Prorodon griseus* (TANNREUTHER, 1926), *Dallasia frontata* (CALKINS and BOWLING, 1929) and the Ophryoscolescidae (DOGIEL, 1925).

Second maturation division.

The second maturation division somewhat resembles the vegetative division. The micronuclei resulting from the first division exhibit a condition in which the chromatin is in the form of long threads, somewhat tangled. These threads form into sixteen long chromosomes, oriented in one direction (Pl. 5 Fig. 6). The nucleus, measuring 14 μ to 16 μ in diameter, elongates slightly while the

chromosomes shorten to form a definite metaphase plate (Pl. 5 Fig. 7). I see no evidence of pairing of the chromosomes, as described in *Prorodon griseus* (TANNREUTHER, 1926) and *Euplotes patella* (TURNER, 1930). The resulting anaphase shows two sets, each with eight ribbon-like chromosomes (Pl. 5 Fig. 8). These chromosomes fuse and result in two heavily staining masses of chromatin (Pl. 5 Fig. 9). This, as is the usual case, is the reduction division, the normal diploid number of sixteen chromosomes (found in normal vegetative division) being reduced to eight. The daughter nuclei now go into a resting condition, remaining relatively small, 7 μ to 10 μ in diameter.

Third maturation division.

The third and last maturation division is characterized, as in *Uroleptus halseyi* (CALKINS, 1930 b), by an immense increase in the staining reaction of the chromosomes. Eight large, even chromosomes are formed and line up on the spindle, parallel to its long axis (Pl. 5 Fig. 10). These chromosomes are very distinct and accurate counts can be made. The telophase of this division is characterized by a much drawn out appearance. The final separation of the daughter nuclei results in the pronuclei. These pronuclei are in the form of elongate clubs of faintly staining chromatin surrounded by a clear area (Pl. 5 Fig. 11). There seems to be practically no size difference in the four pronuclei of a conjugating pair. I have never observed the actual exchange of the pronuclei, although several cases were found where one pronucleus in each conjugant was near the protoplasmic bridge and separated from its sister pronucleus by quite a distance.

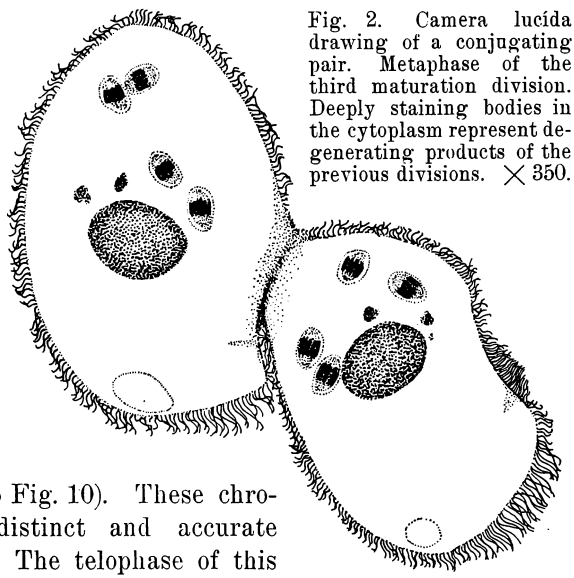


Fig. 2. Camera lucida drawing of a conjugating pair. Metaphase of the third maturation division. Deeply staining bodies in the cytoplasm represent degenerating products of the previous divisions. × 350.

Presumably the conjugants separate very shortly after pronuclear exchange.

The exconjugant.

Many cases of single organisms, undoubtedly exconjugants, containing two typical pronuclei have been found (Pl. 5 Fig. 12). This indicates that separation of the conjugants must take place very shortly after the exchange of the pronuclei. I was unable to find any unquestionable case of the pronuclei in the actual process of fusion. The fact that exconjugants, newly separated, resemble in life the normal vegetative forms makes this stage elusive and its demonstration the result of chance.

Amphinuclear divisions.

Four divisions of the amphi- or zygote nucleus follow in rapid succession resulting in sixteen apparently identical products.

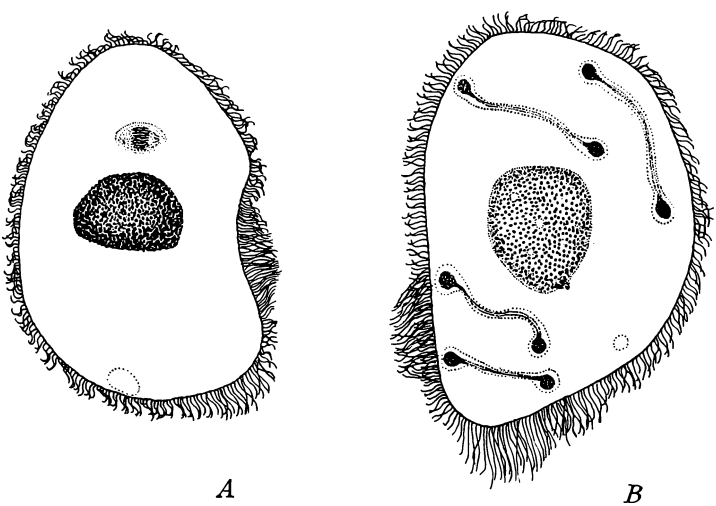


Fig. 3. Exconjugants: A. Metaphase of the first division of the amphinucleus. B. Telophase of the third division of the amphinucleus. Camera lucida. × 350.

The first division of the amphinucleus roughly resembles the second maturation division. The chromosomes are more dense, however, and appear a bit more ragged. Sixteen chromosomes can be counted on the spindle (Text-Fig. 3 A, Pl. 5 Fig. 13). The picture presented is quite different from that of ordinary vegetative division. The mitotic spindle of the first amphinuclear division is found in the cytoplasm anterior to the old macronucleus, while in ordinary binary fission the dividing micronuclei are invariably found in the central cytoplasm lateral to the macronucleus. The structure of the old macronucleus of the exconjugant easily distinguishes it from

the dividing macronucleus. The possibility of confusing the amphinuclear divisions with ordinary binary fission is thus precluded.

The three other divisions of the products of the first amphinuclear division follow quickly. The telophase of the third division is shown in Text-Fig. 3 B, and the sixteen products of these four divisions are all of equal size. They are compact spheres of chromatin, each with a nuclear membrane, greatly resembling the resting micronuclei of the vegetative ciliate (Text-Fig. 4 A, Pl 7 Fig. 32).

Differentiation of macronuclear anlagen.

Striking differentiation results in the formation of from twelve to fifteen macronuclear anlagen and from four to one micronuclei, respectively, none of which degenerates.

The first evidence of differentiation is the swelling and loss of staining capacity on the part of some of the sixteen elements. The remaining ones swell slightly and become a bit more dense (Text-Fig. 4 B).

No clue is given as to just which of the sixteen elements will form micronuclei until swelling of the macronuclear anlagen starts. The determining factor may possibly be in the location in the cell, as the majority of micronuclei are found in the anterior half at this stage. Diller (1928) ascribes such a reason to micronuclear differentiation in *Trichodina* during endomixis. But many of the elements which develop into macronuclear anlagen, in the exconjugant of *Conchophthirius mytili*, are always in the same immediate vicinity.

It is this stage of differentiation that gives rise to the variable number of micronuclei found in vegetative individuals. The combinations found here are entirely consistent with the counts made of micronuclear number in the first of this series of studies.

Fate of the old macronucleus.

Shortly after the union of the conjugants the old macronucleus usually rounds up somewhat. In some cases it becomes slightly evacuolated about the periphery. Very little change is seen from that stage until the start of the differentiation of the new macronuclear anlagen in the exconjugant. The old macronucleus then becomes coarsely granular, retaining, however, its staining capacity (Text-Fig. 4 B). Rapid degeneration follows, the chromatin disappearing within the vesicle of the macronuclear membrane. The

granules get smaller and the mass decreases until all trace of the old macronuclear chromatin and the vesicle has gone.

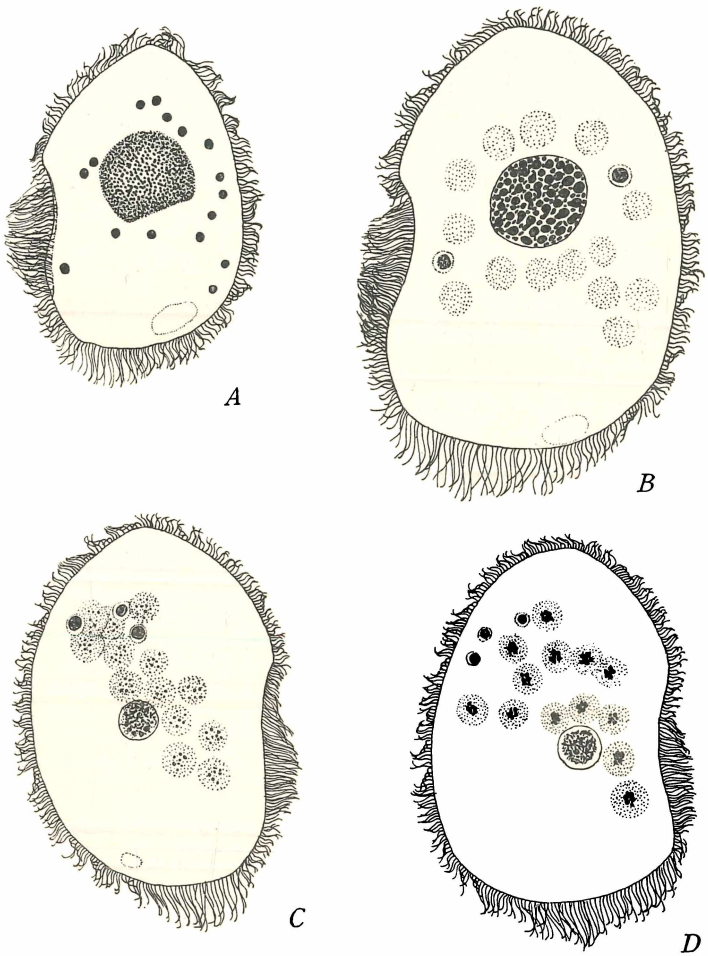


Fig. 4. Exconjugants: A. Completion of the fourth amphinuclear division. Sixteen equal elements and the old macronucleus. B. Differentiation of macronuclear anlagen (fourteen). The old macronucleus is beginning to degenerate. C. Extrusion spheres forming in the macronuclear anlagen (thirteen). The old macronucleus is being absorbed. D. Later stage in the growth of the extrusion spheres. Camera lucida. × 350.

This degeneration is quite different from that usually found in ciliates, both as to time and form. In *Paramaecium* the old macronucleus of the conjugant fragments into ribbons long before separation.

General reorganization of the exconjugant.

Text-Fig. 5 M—U, represents diagrammatically a typical case of reorganization of an exconjugant in which thirteen macronuclear anlagen and three micronuclei have been differentiated. The micronuclei of the exconjugant undergo typical mitosis at each subsequent division, in appearance identical to the vegetative mitoses. The thirteen macronuclear anlagen segregate into two groups, one containing seven and the other six (N). Reorganization of the cytoplasmic structures takes place as in vegetative division. The organism divides, the one daughter receiving seven macronuclear anlagen and three daughter micronuclei, while the other daughter organism receives but six macronuclear anlagen and three daughter micronuclei (O). A second division (P) results in four ciliates, all with the usual three micronuclei. Three of these products of the second exconjugant division have three macronuclear anlagen while the fourth has four. This last is the result of the segregation of the anlagen in the "seven" type (Q). The third division segregates the macronuclear anlagen further (R), the result being eight organisms, three of which have reached the final condition of one macronuclear anlage while the other five still contain two each (S). These last five must divide again (T) before the final condition is reached (U).

The other combinations follow the same general plan of reorganization. The fourteen macronuclear anlagen and the two micronuclei occur about as often as the one given above.

Irregularities of segregation often occur. Segregation from thirteen into five and eight macronuclear anlagen was noted. Cases of second divisions of "seven" types sometimes give rise to daughters containing five and two anlagen respectively. Other irregularities of segregation also occur but are the exception to the general rule. The majority, by far, segregate the anlagen into two equal, or nearly equal, groups at division.

Judging from the numbers of occurrences at any one time, the actual segregation of anlagen and the division of the exconjugant must be a comparatively rapid process while the inter-division period is extremely long. This may possibly be explained by the fact that some time is needed for the elaborate process of chromatin differentiation that takes place in the macronuclear anlagen, which process will now be described.

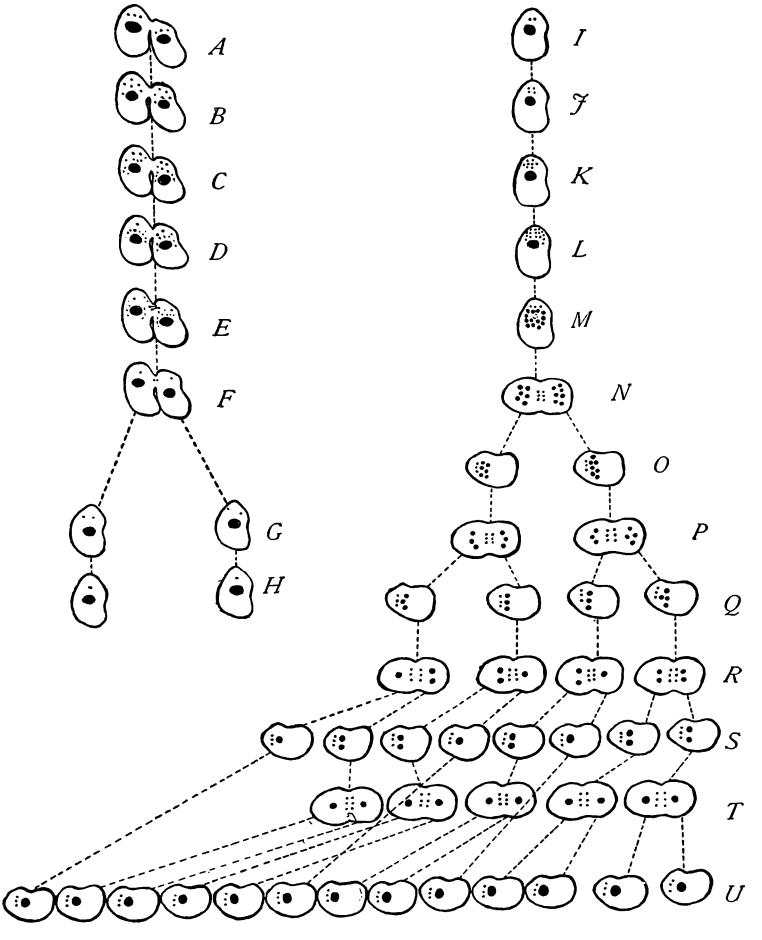


Fig. 5. Diagram of nuclear behavior during conjugation and reorganization. Two tri-micronucleate organisms fuse (A), the micronuclei divide three times (B—D). Due to degeneration of varying numbers of the products of these divisions only two pronuclei are formed (E). There is an exchange of pronuclei. The ciliates then separate (G). The migratory and the stationary pronuclei fuse (H) forming the amphinucleus. This divides four times (I—L) forming sixteen equal products. Thirteen (in this case) differentiate into macronuclear anlagen while three remain as the functional micronuclei. The old macronucleus degenerates (M). The micronuclei divide by mitosis and the anlagen are separated into two groups, one of seven and the other six (N). The exconjugant divides (O). The two resulting ciliates divide a second time with further segregation of macronuclear anlagen (P—Q). A third division results in three ciliates with the final nuclear complex while five still contain two macronuclear anlagen (R—S). The final division of the five (T) results in thirteen ciliates each with one macronucleus and three micronuclei (U).

History of the macronuclear chromatin.

The fourth division of the amphinucleus results in sixteen apparently identical sister nuclei. These nuclei measure about 5 μ in diameter. The FEULGEN nuclear reaction shows them to be made up of homogeneous chromatin. They stain a bright red with the BORREL stain and an intense black with iron alum-haematoxylin. Those that are to become macronuclear anlagen swell to about three times their original diameter with an accompanying loss of staining capacity (Text-Fig. 4 B). The FEULGEN reaction shows them as extremely pale spheres made up of exceedingly minute granules of chromatin (Pl. 6 Fig. 14). The BORREL stain presents a striking picture. The matrix of the sphere is stained a vivid green by the acid component. Throughout this green matrix are scattered the chromatin granules, stained a vivid red (Pl. 6 Fig. 22). As the old vegetative macronucleus begins its degeneration the chromatin content of the anlagen increases and small spheres of dense chromatin begin to collect in their centers (Text-Fig. 4 C). These stain strongly with the FEULGEN reaction (Pl. 6 Fig. 15). The central granules number from six to ten. They soon increase astonishingly in size and staining capacity (Text-Fig. 4 D, Pl. 7 Figs. 34 and 35). There is no evidence that this increase is at the expense of the dispersed chromatin granules of the anlagen as these stain quite evenly throughout (Pl. 6 Figs. 16 and 23). In many cases the picture of this stage reminds one of the metaphase plate of insect spermatocytes, as seen in polar view. When the central granules have reached their maximum density each anlage contains many more times the amount of chromatin than its sister elements that form the micronuclei, if one is to judge by the FEULGEN reaction.

First division of the exconjugant.

As the micronuclei prepare for mitosis a most astonishing thing takes place. The dense central granules within each macronuclear anlage migrate to one edge and come to occupy a somewhat clear area very near the nuclear membrane (Text-Fig. 6, Pl. 7 Fig. 36). After the FEULGEN reaction the remaining chromatin of the anlage appears slightly evacuolated and uneven as if it had been violently stirred (Pl. 6 Fig. 17). The BORREL stain shows the presence of a few green granules scattered among the dispersed red granules of the anlagen (Pl. 6 Fig. 24). At the same time as the chromosomes of the micronuclei are lining up on the spindle, the nuclear membrane of the anlage in contact with the large granules of chromatin

pouches out, carrying with it the whole group of large granules (Text-Fig. 7 A). All of the anlagen seem to act in unison. Within the anlage at the base of the cone formed by the outpouching membrane, a large clear space is seen after the FEULGEN reaction (Pl. 6 Fig. 18). Careful scrutiny reveals this space as a vacuole containing a colorless, granular sphere. The BORREL stain shows this sphere to react intensely within the green acid component (Pl. 6 Fig. 25). It is retained within the nucleus along with a few other green granules. As the micronuclei reach the telophase stage the cones have constricted and broken, leaving the masses of solid chromatin lying in the cytoplasm (Text-Fig. 7 B, Pl. 7 Fig. 37). Here they begin to disintegrate as the first division of the exconjugant is nearly complete. Text-Fig. 8 A shows a posterior daughter of the first division. Reorganization of the anterior cilia is not yet complete. In the cytoplasm may be seen dim fragments of the seven masses of chromatin that have been extruded from the seven macronuclear anlagen during the previous division.

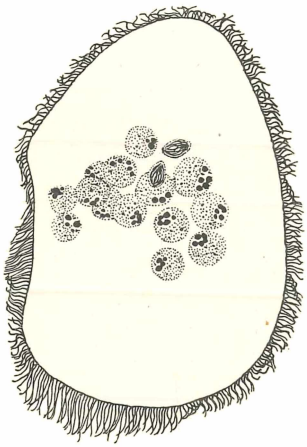


Fig. 6. Exconjugant: Micronuclei in prophase of first exconjugant division. Extrusion spheres migrating to the periphery of the macronuclear anlagen prior to being cast into the cytoplasm. Camera lucida. × 350.

Second division of the exconjugant.

During and after the first division of the exconjugant the anlagen undergo another period of differentiation. One to three small chromatin spheres form, not in the center as in the previous stage, but very near the periphery of each anlage. The achromatin spheres, that formed just prior to the chromatin extrusion, become somewhat dispersed. After the FEULGEN reaction the small spheres are a deep lavender while many small colorless spheres appear in vacuoles (Pl. 6 Fig. 19). The BORREL stain shows these scattered spheres to react to the green acid component. The chromatin spheres near the periphery are stained a bright red (Pl. 6 Fig. 26). During this second period of differentiation the anlagen have increased slightly in diameter and the granular structure as a whole has increased in staining capacity.

The micronuclei again divide and at the same time the second set of chromatin spheres are extruded (Text-Fig. 8 B). This extrusion is accomplished much after the manner of the first, the main difference being that only a small proportion of chromatin bulk is thrown out. Large, green spheres are again formed in the anlagen (Pl. 6 Fig. 27). Upon division of the cell the extruded chromatin quickly disappears in the cytoplasm.

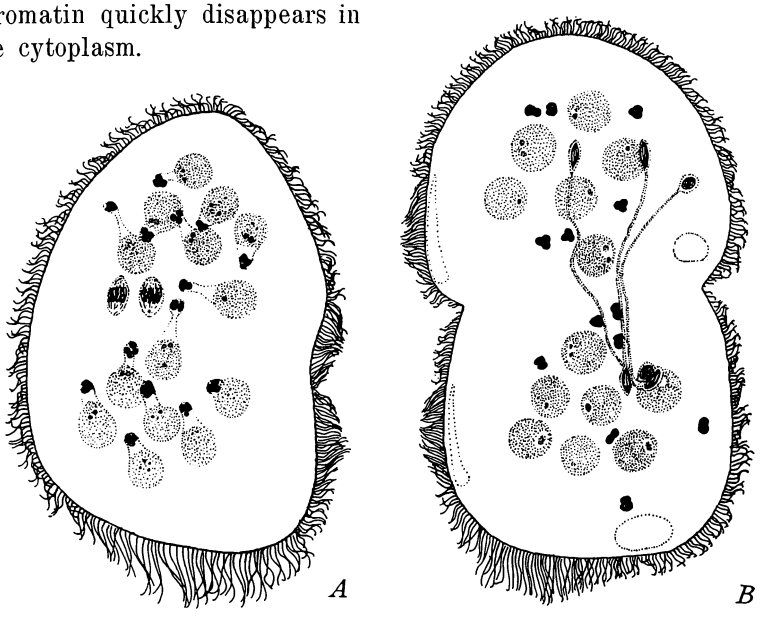


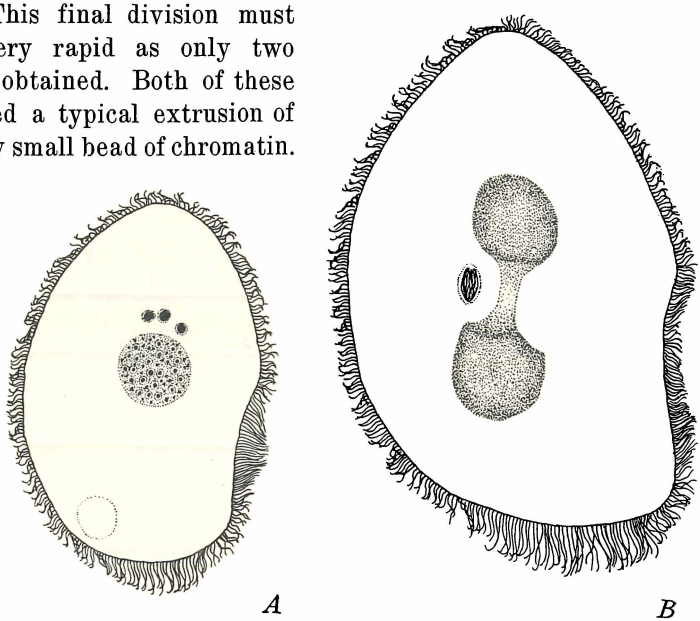
Fig. 7. Exconjugants: A. Macronuclear anlagen have segregated into two groups of seven each and are casting out the ex- trusion spheres. The micronuclei are in full metaphase. B. First division of the exconjugant. The micronuclei are in late telophase. In this case there is an unequal distribution of macronuclear anlagen (seven and six). The extrusion spheres are seen in the cytoplasm, where they will disintegrate. Camera lucida. × 350.

Third and fourth divisions of the exconjugant.

The remaining three (rarely four) anlagen increase markedly in size and differentiate the third set of chromatin spheres to be extruded. These are still smaller than the preceeding ones, occupying, however, the same relative position near the periphery. Numerous achromatin spheres are also present (Text-Fig. 8 C, Pl. 6 Figs. 20 and 28). The usual micronuclear division ensues and the third set of chromatin granules coalesce and are extruded. Pl. 6 Fig. 21 shows the extrusion of the comparatively small amount of chromatin, as seen after the Feulgen reaction. The division of the cell (Text-

Fig. 8 D Pl. 7 Fig. 38) results in one daughter organism possessing the final nuclear combination, one macronucleus and one to four micronuclei. The other daughter organism must divide again.

This final division must be very rapid as only two were obtained. Both of these showed a typical extrusion of a very small bead of chromatin.



A *B*

Fig. 8. Exconjugants: A. Posterior daughter of a first exconjugant division. The anterior cilia have not reorganized. The second set of extrusion spheres are forming in the macronuclear anlagen. The first extrusion spheres have nearly disappeared in the cytoplasm. B. Second division of the exconjugant. This organism contains eight macronuclear anlagen, the result of an unusual distribution at the first division. Second set of extrusion spheres being cast into the cytoplasm. C. Result of the second exconjugant division. Three macronuclear anlagen and four micronuclei. The third set of extrusion spheres are forming. D. Third division of the exconjugant. Small buttons of chromatin have been cast into the cytoplasm. The posterior daughter of this division will contain the final nuclear complex. Camera lucida. × 350.

Growth of the new macronucleus.

The final division leaves the organism with a single macronuclear anlage (Text-Fig. 9 A) approximately one half the size of the normal vegetative macronucleus. It is composed of a matrix, lacking in ability to react to the basic stains, in which are embedded countless granules of chromatin. The matrix is stained green by the BORREL stain.

As the anlage increases in diameter islands of chromatin make their appearance (Text-Fig. 9 A). These react intensely with the

Feulgen reaction and are stained a bright red with the Borrel stain. They appear to lie in vacuoles in the matix (Pl. 6 Fig. 29). These

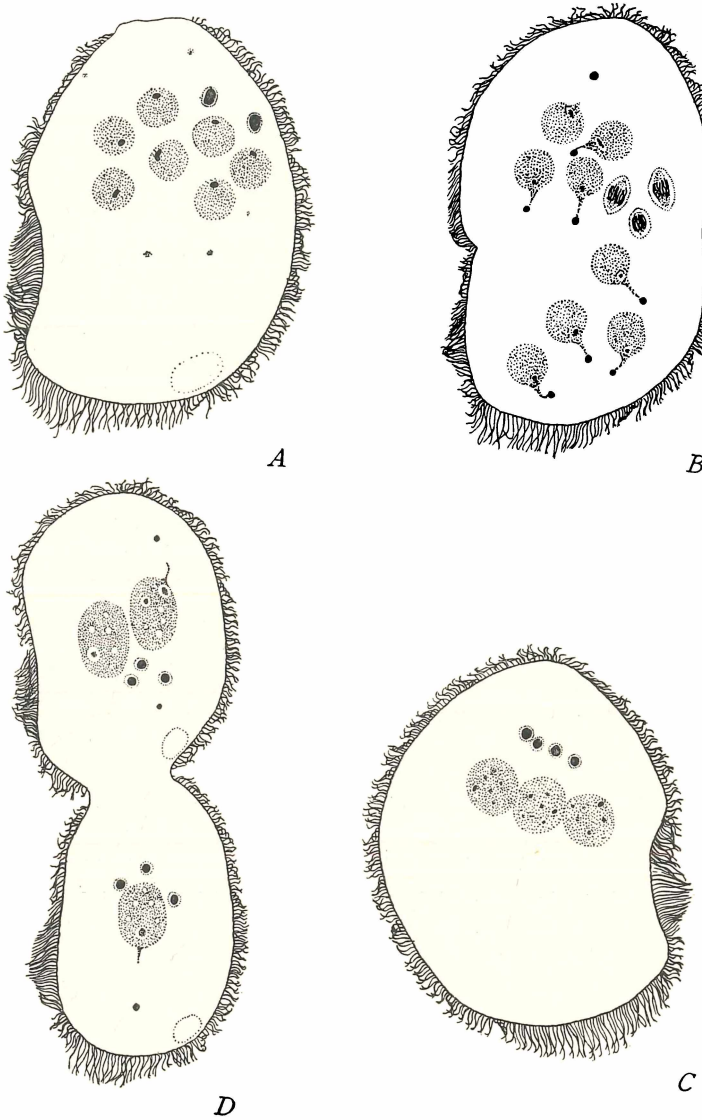


Fig. 9. A. Growth of the new macronucleus. Chromatin islands in the achromatin matrix. B. Division of a newly reorganized ciliate. The daughter macronuclei ar separating cleanly. Camera lucida. × 350.

spheres of chromatin enlarge and gradually replace the matrix, much the same as happens in the "placenta" of *Uroleptus halseyi* (Calkins,

1930 a). By the time the chromatin granules have come to fill the membrane the whole has reached a size of approximately that of the macronucleus of a normal vegetative individual.

Division of reorganized organism.

Subsequent divisions of the reorganized ciliates follow the course taken in normal vegetative division, with one important difference. No residual chromatin is left behind as the two daughter macronuclei pull apart. Instead of having the peculiar ball of chromatin left in the separation plane, as was described in the first paper of this series, the two daughter macronuclei separate cleanly (Text-Fig. 9 B). This situation continues for a little over a month, according to my observations, and then the residual chromatin again begins to appear. At first the amount is small but within a short time it is as large and as regular in occurrence as ever.

One can readily see that the actual elapse of time between the reorganization and the first appearance of the residual mass of chromatin can be only roughly approximated, since I have had no success in keeping the ciliate alive outside the host.

Discussion.

The peculiar mode of attachment during conjugation might be explained by the form and habits of *Conchophthirius mytili*. This ciliate is quite flat and is always found with its concave surface in contact with the substrate. Only occasionally does it swim free in the water of the syracuse dish, and probably not at all in its natural environment. If we can assume this situation to be true then the only manner whereby two ciliates might fuse by their oral regions would be a head-on approach. In other words the conjugants would be progressing in opposite directions. The only other means would be for one of the ciliates to leave the substrate and turn with its ventral side up, as seems to be the case with *Chilodon* (MACDOUGALL, 1925) and *Ancistrum mytili* (work now in progress). Fusion as it occurs, however, can be accomplished while both ciliates are creeping in the same direction, ventral surfaces in contact with the substrate.

In as much as the micronuclei of *Conchophthirius mytili* follow the regular procedure of ciliate micronuclei during conjugation, little need be said in that connection. Reduction appears to take place during the second maturation division where the diploid number of sixteen chromosomes results in two sets of eight each. The logical method of this halving would seem to be a sorting out of the two

sets of eight from the plate of sixteen on the second maturation spindle. This conception falls in line with the explanation given by TURNER (1930) for the regular appearance of twice (or four times in *Euplotes*) the number of granules on the metaphase plate of the first maturation spindle, as the diploid chromosome count. He assumes this number to be the result of prophase separation of the chromatids. My observations are entirely consistent with this explanation. The sixteen chromosomes of the vegetative micronuclei appear to be halved into thirty-two chromatids. No union into tetrads occurs. Sixteen chromatids pass to each daughter nucleus at the first division. Without further separation these sixteen chromatids line up on the second maturation spindle, and a second segregation takes place, resulting in two groups of eight each.

The four equal divisions of the amphinucleus are uncommon. PROWAZEK (1899), in *Bursaria truncatella*, describes sixteen nuclei being formed from the divisions of the amphinucleus. In *Bursaria*, however, only two to five become macronuclei and three or more become micronuclei, while the rest degenerate.

POLJANSKY (1928) re-investigated the conjugation of *Bursaria truncatella* and found that the macronuclear anlagen are differentiated after the third amphinuclear division instead of after the fourth.

In *Conchophthirius mytili* all sixteen products of the four amphinuclear divisions have a future. This reminds one of the reorganization of *Trichodina* during endomixis as described by DILLER (1928). Only three nuclear divisions take place in this form, but of the eight products one becomes a micronucleus while the other seven enlarge to form macronuclear anlagen. No degeneration occurs. The seven macronuclear anlagen are segregated out into seven daughter organisms at subsequent divisions, while the micronucleus undergoes mitosis. During the differentiation of these macronuclear anlagen a loss of "chromaticity" occurs. DILLER describes this as happening by the extrusion of chromatic granules from the surface of the anlagen, or by "autodigestion" of chromatic material effected within the anlagen.

In this discussion I am using the term "chromatin" to represent the material which reacts to give a lavender color after the FEULGEN reaction. The FEULGEN reaction, however, indicates the presence of nucleic acid (thymonucleic acid, STEUDEL, 1912) and the coloration depends on the acid hydrolysis of the nucleic acid molecule. According to FEULGEN and ROSSENBECK (1924) the purins, guanin and adenin are split off and form a reducing group. These react after

the manner of an aldehyde and are colored a vivid lavender by the basic fuchsin in the presence of HCl and the sodium bisulphite. This is SCHIFF's aldehyde reaction. For a detailed account of the chemistry of this reaction in relation to thè cell nucleus see PRATJE (1920), FEULGEN and ROSSENBECK (1924) and ROBERTSON (1927).

The term "chromatin" has been used in a more or less general sense, as is pointed out by CALKINS (1930 a) and there is the possibility that chromatin may represent a nucleo-protein having nucleic acid as a variable component. This would appear to be the case in the "placenta" of *Uroleptus halseyi* where the nucleic acid content diminishes and then reappears. However my use of the term "chromatin" as synonymous with "nucleic acid" is presented in lieu of an adequate definition of chromatin.

The meaning of the regular extrusion of chromatin from the macronuclear anlagen in *Conchophthirius mytili* is speculative. It may represent the sloughing off of the germinal chromatin contained in the amphinucleus, a substance that is superfluous for the further activity of a purely trophic cell element (REICHENOW, 1927). This was suggested by DILLER (1928) as the explanation of the extrusion of chromatic granules from the macronuclear anlagen of *Trichodina*. If this is the case then the extrusion chromatin of *Conchophthirius mytili* represents not only the germinal chromatin but also an immense amount of accumulated substance which reacts in every way as chromatin. The amount of material extruded from one anlage is many times that found in the micronuclei.

Another possible explanation of the extrusion of chromatin, consistent with the above hypothesis, may be as follows. In most cases of exconjugant reorganization reported in ciliates one or more of the products of the amphinuclear divisions disintegrate in toto. This may indicate that segregation of materials no longer needed by the macronucleus has taken place very early, and the daughter nuclei containing this material are no longer capable of development. In *Uroleptus mobilis* (CALKINS, 1919) the second amphinuclear division is heteropolar, the large daughter forming the new macronucleus and the small daughter disintegrating. If one is to extend the analogy to the case of *Conchophthirius mytili* the macronuclear anlagen are comparable to the new macronucleus of *Uroleptus* while the extrusion chromatin is comparable to the smaller disintegration nucleus. In that light one might consider the phenomenon here observed as a heteropolar division of the macronuclear anlagen

keeping pace with the micronuclear divisions, the smaller products destined to disintegrate.

The formation of the new macronucleus appears to be in the same manner as that described by CALKINS (1930 a) for *Uroleptus halseyi*. The chromatin granules enlarge until they replace the achromatin matrix. He calls this increase of chromatin "actual manufacture" occurring in the matrix.

Summary.

1. The subject of this study is *Conchophthirius mytili*, a ciliate commensal in the salt water mussel, *Mytilus edulus*.

2. Conjugation probably occurs but rarely, one epidemic being noted. At conjugation a large ciliate fuses at the anterior end of the peristomal groove with the anterior aboral surface of a slightly smaller organism.

3. The number of micronuclei in the conjugants may be equal or unequal.

4. Three maturation divisions take place, the second of which appears to result in reduction of the chromosome number from sixteen to eight. Two pronuclei are formed in each conjugant as the result of the third maturation division.

5. The conjugants separate shortly after pronuclear exchange and before pronuclear fusion has taken place.

6. The amphinucleus divides four times giving rise to sixteen apparently equal products. Differentiation results whereby fifteen to twelve enlarge and become macronuclear anlagen, the remaining one to four becoming the functional micronuclei.

7. The old macronucleus begins disintegration during differentiation of the macronuclear anlagen.

8. The macronuclear anlagen are segregated out during subsequent cell divisions until the final number of one is contained within each daughter organism. The micronuclei, meanwhile, undergo typical mitoses at each exconjugant division.

9. Chromatin spheres become collected in each macronuclear anlage prior to divisions and are cast out into the cytoplasm where they disintegrate.

10. The new macronucleus is elaborated at the close of the segregation divisions by an increase in the deeply staining chromatin granules, these ultimately replacing the achromatin matrix of the anlage.

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Explanation of Plates.

Plate 5—7.

All figures are of *Conchophthirius mytili*. Pl. 1—2 drawn with the aid of camera lucida from fixed material. Figures of Pl. 3 are photomicrographs taken with 4 mm Spencer and 1.5 mm Koristka apochromatic objectives, with 5 × and 10 × oculars. Abbreviations for fixatives: B. Bouin's; G.-C. Gilson-Carnoy's; S.-A.-Alc. sublimate-acetic in 95% alcohol; Sch. Schaudinn's. Abbreviations for stains: Bor. Borrel; Del. Delafield's haematoxylin; Feul. Feulgen nuclear reaction; Heid. Heidenhain's haematoxylin.

Plate 5.

Fig. 1. Early prophase of first maturation division. Chromatin clumped in center of swelling micronucleus. G.-C., Heid. × 1500.

Fig. 2. Prophase of first maturation division. Chromatin spreading out in a plate on the spindle. Sch., Heid. × 1500.

Fig. 3. Metaphase of first maturation division. Thirty-two rough "chromosomes" at equatorial plate. Sch., Heid. × 1500.

Fig. 4. Early anaphase of first maturation division. "Chromosomes" have segregated into two groups of sixteen each. Sch., Heid. × 1500.

Fig. 5. Late anaphase of first maturation division. B., Heid. × 1500.

Fig. 6. Late prophase of second maturation division. Sixteen long chromosomes oriented in one direction. G.-C., Feul. × 1500.

Fig. 7. Metaphase of second maturation division. Chromosomes have shortened and spindle fibers are visible. G.-C., Feul. × 1500.

Fig. 8. Late anaphase of second maturation division. Eight chromosomes appear at each pole of the spindle. G.-C., Heid. × 1500.

Fig. 9. Early telophase of second maturation division. Chromatin fused and condensed. G.-C., Feul. × 1500.

Fig. 10. Metaphase of third maturation division. Eight large, deeply staining chromosomes on spindle. Sch., Del. × 1500.

Fig. 11. Two pronuclei of one conjugant. Sch., Del. × 1500.

Fig. 12. A single exconjugant with the old macronucleus and two pronuclei, prior to amphinuclear formation. S.-A.-Alc., Feul. × 250.

Fig. 13. Metaphase of first amphinuclear division. Sixteen well formed chromosomes on a pointed spindle. S.-A.-Alc., Heid. × 1500.

Plate 6.

Figs. 14—29 represent the details of the nuclear reorganization in the exconjugant. × 1010.

Fig. 14. One macronuclear anlage and one micronucleus in an early stage of differentiation. G.-C., Feul.

Fig. 15. Early stage in the formation of the extrusion spheres. G.-C., Feul.

Fig. 16. The extrusion spheres have enlarged and the whole anlage has increased in staining capacity. G.-C., Feul.

Fig. 17. Micronucleus in prophase of mitosis. Extrusion spheres have migrated toward the periphery of the anlage. Note the appearance of the remaining chromatin. Sch., Feul.

Fig. 18. Micronucleus in full metaphase of first exconjugant division. Spheres are being cast out. Vacuoles appear among the remaining chromatin granules. SCH., FEUL.

Fig. 19. Macronuclear anlage after the first exconjugant division. This is one of a group of six. The second set of extrusion spheres are collecting, this time near the periphery. Compare with Text-Fig. 8 A. SCH., FEUL.

Fig. 20. Macronuclear anlage after the second exconjugant division. The third set of extrusion spheres are very small. The anlage has swollen. Compare with Text-Fig. 8 C. SCH., FEUL.

Fig. 21. Macronuclear anlage during the third exconjugant division. The extrusion spheres have fused into a small button of chromatin and are being cast out into the cytoplasm. Compare with Text-Fig. 7 D. SCH., FEUL.

Fig. 22. BORREL stain of a macronuclear anlage in the same stage as Fig. 14. The matrix is a vivid green through which are scattered the bright red chromatin granules. SCH., BOR.

Fig. 23. Elaboration of the extrusion spheres prior to the first exconjugant division. Compare with Fig. 16. SCH., BOR.

Fig. 24. Migration of spheres as in Fig. 17. A few green granules are beginning to appear. These correspond to the vacuoles seen after the FEULGEN reaction. SCH., BOR.

Fig. 25. A BORREL stain in which the differentiation of the red, basic stain was prolonged. The red has completely disappeared from the chromosomes of the micronucleus and the fine granules of the macronuclear anlage. The extrusion spheres are still a brilliant red. Many green spheres in the anlage. SCH., BOR.

Fig. 26. A BORREL stain of a macronuclear anlage in same stage as Fig. 19. SCH., BOR.

Fig. 27. A micronucleus in full mitosis during the second exconjugant division. One macronuclear anlage of a set of eight. The second set of extrusion spheres have fused and are being cast out. Large green spheres retained in the anlage. SCH., BOR.

Fig. 28. A BORREL stain of a macronuclear anlage in same stage as the one shown in Fig. 20. S.-A.-Alc., BOR.

Fig. 29. A stage in the growth and elaboration of the new macronucleus. Many large chromatin islands have formed in the green, achromatin matrix. Compare with Text-Fig. 9 A. SCH., BOR.

Plate 7.

Photomicrographs.

Fig. 30. Ventral view of conjugating pair. Each conjugant has two micronuclei in early anaphase of first maturation division. Note larger size of right hand organism. S.-A.-Alc., HEID. × 314.

Fig. 31. Same. Enlarged view of micronuclei of larger conjugant. × 1040.

Fig. 32. Ventral view of exconjugant after fourth amphinuclear division. Sixteen equal elements may be seen surrounding the still intact old macronucleus. See Text-Fig. 4 A. SCH., BOR. × 265.

Fig. 33. Ventral view of exconjugant after differentiation of macronuclear anlagen and disintegration of old macronucleus. This individual contains three micronuclei and thirteen macronuclear anlagen. SCH., DEL. × 265.

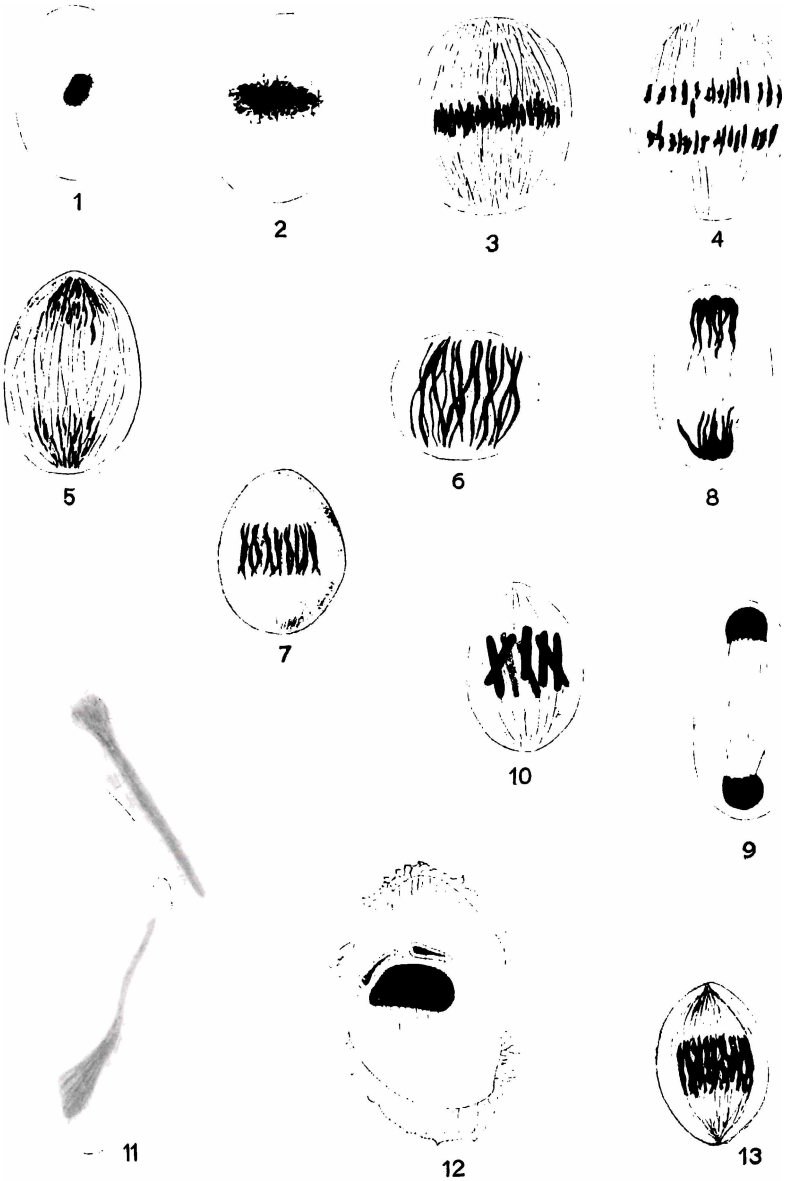
Fig. 34. Ventral view of exconjugant. Fourteen macronuclear anlagen, each containing a group of large extrusion spheres. Two deeply staining micronuclei. Food inclusions in posterior cytoplasm. S.-A.-Alc., Bor. × 597.

Fig. 35. Enlarged view of the extrusion spheres before first exconjugant division. Sch., Bor. × 1715.

Fig. 36. Migration of extrusion spheres just prior to first exconjugant division. One micronucleus in late prophase shown. Sch., Feul. × 1715.

Fig. 37. Ventral view of posterior daughter during first exconjugant division. Plasmotomy not complete. The anterior daughter has seven macronuclear anlagen while the one shown has six. Three daughter micronuclei may be seen dimly to the upper right of the macronuclear anlagen. Five extrusion masses are in sharp focus while the sixth resembles the micronuclei. At the extreme upper right an extrusion mass from one of the anterior group may be seen. Sch., Feul. Borrel II counterstain. × 597.

Fig. 38. Ventral views of one exconjugant taken at different foci. The third division is under way. Small beads of chromatin are being thrown out into the cytoplasm. Sch., Del. × 265.



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VERLAG VON GUSTAV FISCHER IN JENA.

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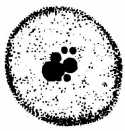

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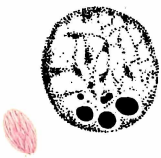

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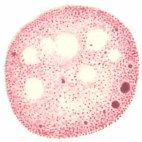

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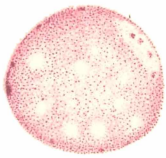

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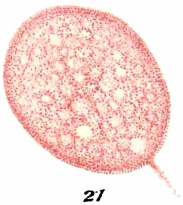

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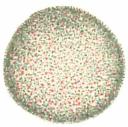

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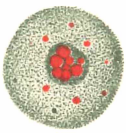

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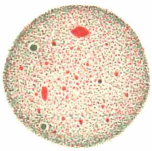

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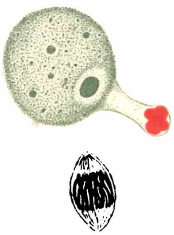

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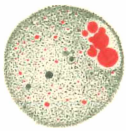

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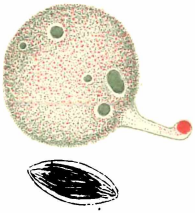

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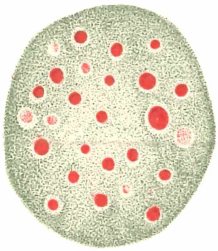

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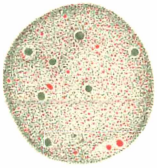

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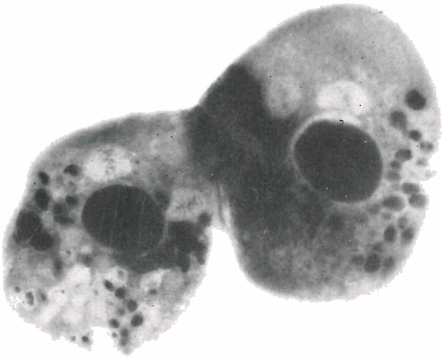

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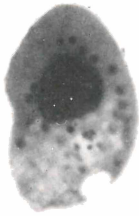

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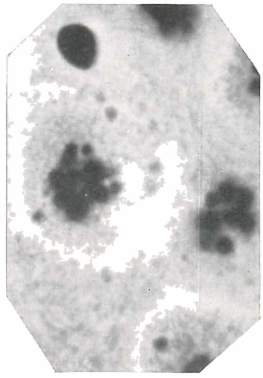

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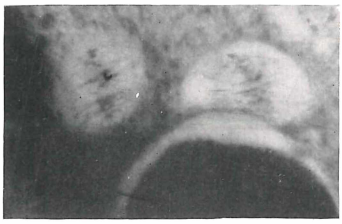

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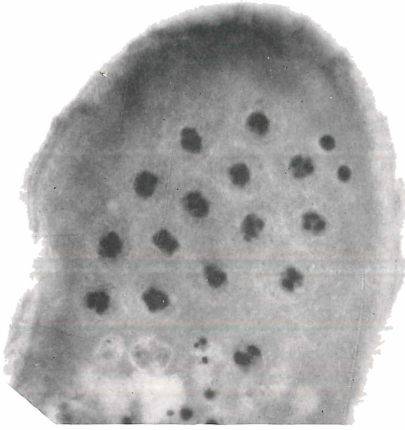

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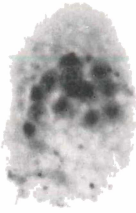

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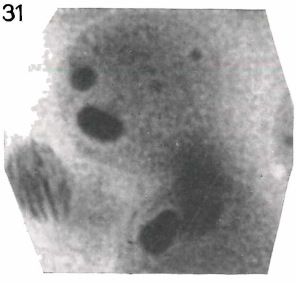

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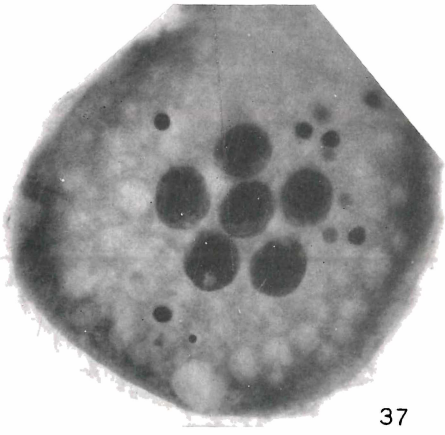

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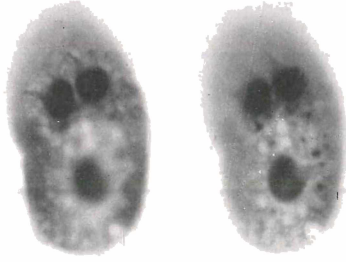

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